

## GENETIC MODIFICATION OF THE DILATED CARDIOMYOPATHY AND NEONATAL LETHALITY PHENOTYPE OF MICE LACKING MANGANESE SUPEROXIDE DISMUTASE

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Oxygen radical-mediated tissue damage has been implicated in a large number of pathological conditions including ischemia reperfusion injury (Chan 1994, Euler 1995, Kinouchi et al. 1991, Samaja et al. 1994), neurodegenerative diseases (Fahn and Cohen 1992, Jenner 1994, Lafon-Kazal et al. 1993), neonatal hyperoxic lung injury (Davis et al. 1993), atherosclerosis (Halliwell 1993), and the aging process (Ku et al. 1993, Orr and Sohal 1994). Therefore, many studies have been focused on enzymes and molecules that can scavenge oxygen radicals for their potential in the prevention and/or therapy of these disorders.

Under normal circumstances, the majority of oxygen radicals are generated in the mitochondria as a byproduct of electron transport and oxidative phosphorylation required for the production of ATP. Cells are protected against this metabolically-induced oxidative stress by several oxygen radical scavengers, including the superoxide dismutases, catalase, glutathione peroxidase, and reduced glutathione. Among these, one form of superoxide dismutase, manganese superoxide dismutase (MnSOD), has been the subject of particular interest because it is located within mitochondria, can be induced by several cytokines and superoxide itself, and appears to be involved in other processes, including tumor suppression and cellular differentiation (Akashi et al. 1995, Church et al. 1993, Harris et al. 1991, Sato et al. 1995, St. Clair et al. 1994).

To investigate the role of MnSOD and to distinguish its actions from those of cytoplasmic CuZnSOD, the mouse MnSOD gene (*Sod2*) was inactivated by homologous recombination (Li et al. 1995) to delete exon 3. Phenotypic analysis of the mutant animals placed on the CD1 *tm1<sup>ucsf</sup>* (an outbred strain) genetic background (CD1-*Sod2*<*tm1Cje*>, formerly designated as *Sod2*) indicated that whereas there is no detectable atypical phenotype in the heterozygous mutants (*Sod2*+/+) up to 9 months of age, homozygous mutant mice (*Sod2*-/-) die within the first 10 days of life. The major phenotype abnormalities observed with *Sod2*-/- mice include: 1) dilated cardiomyopathy with a thin left ventricular wall and enlarged left ventricular cavity; 2) accumulation of lipid in liver and skeletal muscle; 3) metabolic acidosis with increased ketones and decreased bicarbonate in the plasma; 4) excretion of 3-hydroxy-3-methylglutaric and 3-hydroxy-3-methylglutaconic acids, 5) respiratory alkalosis, as an attempt to compensate for the metabolic acidosis; and 6) a severe reduction in succinate dehydrogenase (com-

plex II) and aconitase (a TCA cycle enzyme) activities in the heart and, to a lesser extent, in other organs. These findings indicate that MnSOD is required for the normal biological function of tissues by maintaining the integrity of mitochondrial enzymes susceptible to direct inactivation by superoxide.

MnSOD mutant mice [designated *Sod2*<sup>*m1BCM*</sup>] have also been generated by Lebovitz et al. (1996) by gene targeting to produce a deletion of exons 1 and 2. Homozygous *Sod2*<sup>*m1BCM*</sup> mutant mice on a mixed genetic background survived for as long as 18 days. These mice are anemic and exhibit progressive motor abnormalities characterized by weakness, rapid fatigue and ataxia. In addition, lack of myelination was observed in the spinal cord, and dystrophic neurons were seen scattered throughout the CNS. Possible explanations for the discrepancy of the phenotypes observed in the two different strains of MnSOD deficient mice include differences between the targeting vectors used to generate the mutations, between the embryonic stem cells employed, and/or between the genetic backgrounds on which the mutant mice were bred. A major influence of genetic background on the mutant phenotype of several strains of knockout mice has been reported (Threadgill et al. 1995; Rozmahel et al. 1996), and these phenotypic differences have become quite important for revealing the existence of interesting genetic modifiers of the effects of the primary mutations. Therefore, *Sod2*<*tm1Cje*> -/- mice were generated on two backgrounds in addition to the CD1 background originally used: 8 to 10 generations of backcrosses to C57BL/6J (designated B6) which made them 99.6 to 99.9% congenic on B6, and B6D2 F2 [the C57BL/6J heterozygotes were crossed to DBA/2 (designated D2) to generate B6D2 F1 animals, and then these F1s were intercrossed to generate B6D2 F2 animals for phenotypic analysis.

In contrast to the 10 and 18 day survival times of CD1-*Sod2*<*tm1Cje*> and *SOD2*<sup>*m1BCM*</sup>, respectively, the live-born -/- mice on C57BL/6J background (B6<*Sod2*-/->) survived for only up to 4 days, with an average life span of 1.5 days. In addition, about half of the B6<*Sod2*-/-> mice died about day 15 of gestation, and the -/- fetuses appeared very pale. On the other hand, the liveborn B6D2 F2<*Sod2*-/-> mice survived for up to 18 days with an average life span of 11 days. The long-lived B6D2 F2<*Sod2*-/-> mice ( $\geq 15$  days) displayed neurological abnormalities which included ataxia and seizures. How-

ever, their hearts were not as severely dilated as those of the CD1<Sod2-/-> mice, and neurological complications, rather dilated cardiomyopathy, appear to be the cause of death in the long-lived B6D2 F2<Sod2-/-> mice. These findings imply the existence of genetic factors that modify the target organ damage in *Sod2-1-* mice, perhaps by modulating of oxygen free radicals. Identification of these genetic modifier(s) will advance our understanding of the mechanisms of oxidative tissue damage and may offer potential strategies for prevention and therapy.

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